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(54) Title: SYNTHETIC IgE MEMBRANE ANCHOR PEPTIDE IMMUNOGENS FOR THE TREATMENT OF ALLERGY

(57) Abstract

The present invention relates to a method for eliciting the production in healthy mammals, including humans, of high titer antibodies specific for sites on the extracellular segment of the anchor domain of the membrane-bound ε heavy chain of B cell-expressed humain IgE by the use of a composition comprising a synthetic peptide immunogen containing extracellular membrane anchor sites, to reduce IgE-secreting B leukocytes and allergen-induced IgE production. It also relates to the use of optimally designed, carrier protein free, IgE ε-chain related immunogens as key components in a synthetic vaccine to provide an immunotherapy for the treatment of allergy. The subject peptides contain immune stimulator sequences, including a tandemly linked helper T cell epitope, to aid in stimulating the immune response towards the mIgE membrane anchor domain.

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SYNTHETIC IgE MEMBRANE ANCHOR PEPTIDE IMMUNOGENS FOR THE TREATMENT OF ALLERGY

FIELD OF THE INVENTION

The present invention relates to the use of a composition of a synthetic peptide immunogen comprising a target antigenic site and a helper T cell epitope covalently linked in a linear tandem form. More particularly, the present invention relates to the use of such a composition to elicit the production in healthy mammals, including humans, of high titer antibodies specific to sites on the ϵ heavy chain of B cell-expressed membrane-bound human IgE, i.e., sites on the extracellular segment of the anchor domain of membrane-bound human ϵ-chain and to the use of such a composition as a vaccine to provide an immunotherapy for the treatment of allergy.

BACKGROUND OF THE INVENTION

Immunotherapy for the prevention of IgE-mediated allergic responses such as asthma and hay fever, as known and practiced since early in this century, has involved desensitization or hyposensitization by administering a gradually increasing amount of an allergen to a patient to reduce the effects of subsequent exposure to that allergen[1]. Limitations to such an allergen-based immunotherapy include difficulties in identifying the allergen involved and the adverse reactions frequently caused by the use of the identified allergen[2]. Other treatments for the relief of allergies employ therapeutic compounds to block the cascade of cellular events that is responsible for allergic reactions. These compounds include anti-histamines, decongestants, β_2 agonists, and corticosteroids. Anti-histamines, decongestants, and β_2 agonists act on events downstream of IgE in the allergic cascade, making them palliative remedies which address allergic symptoms rather than preventative treatments which must act on events closer to the initiation of IgE-

mediated allergic reactions. These palliative remedies provide relief that is short term and partial, frequently accompanied by adverse side effects. For example, anti-histamines may cause restlessness or sedation, and β_2 agonists are sometimes associated with increased morbidity in asthmatic patients.

In contrast to anti-histamines, decongestants and β_2 agonists, corticosteroids are powerful immunosuppressants and are highly efficacious for the treatment of allergic symptoms. However, they produce adverse hormonal activities and may cause an undesirably broad immunosuppression. To avoid the shortcomings of these therapeutic compounds, it would be desirable to prevent allergic responses at the level of IgE, with a means of suppression selectively targeted to IgE. This may be accomplished by suppressing IgE synthesis directly or indirectly. Indirect suppression can be accomplished by desensitization or by inhibition of IL-4 and other T cell-produced mediators of IgE synthesis[3]. Direct suppression, as suggested by Chang et al.[4], can be accomplished by specifically targeting IgE-producing B cells with selective antibodies.

Chang et al.[4-8] and others[9] have studied human ϵ-chains and corresponding antibodies, as well as the genes and mRNAs by which the ϵ-chains are encoded. They have elucidated the molecular basis for the expression of two types of IgE: the secreted and membrane-bound forms by B cells committed to IgE synthesis. The membrane-bound form of IgE (mIgE) may be distinguished from the secreted form by an additional membrane anchoring domain that extends from the C-terminus of the heavy chains and is contiguous with the CH4 constant domain of IgE. The membrane-bound form is distinctive to the surface of B cells committed to IgE synthesis. By targeting such cells with antibodies specific for the exposed extracellular portion of that anchor domain, such cells may be

eliminated or inactivated. The mechanisms for elimination of IgE-secreting cells by such antibodies can be through antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytolysis[6,7]. The reduction of circulating IgE and IgE-expressing cells by anti-IgE antibodies has been demonstrated *in vivo* in mice[10], and by the inhibition *in vivo* of passive cutaneous anaphylaxis in a rat model. It has also been demonstrated in human IgE-secreting cell lines where anti-IgE was shown to lead to reductions in cell growth, decreased IgE accumulation and cytolysis in complement-mediated and ADCC-mediated cytolysis assays[11]. By determining the nucleotide sequences of pertinent segments in human genomic DNA and in mRNA from human mIgE-expressing B cells, amino acid sequences for the extracellular portion of the anchor domain were predicted. The presence and specificity of these sites as well as their accessibility to antibodies were confirmed with specific antibodies[4,8]. These polyclonal and monoclonal antibodies were anti-peptide antibodies derived from immunizations with ε chain-related "peptide-carrier protein conjugate"[4,8]. The carrier protein was Keyhole Limpet Hemocyanin (KLH) known to be useful for its capability of stimulating antibody responses to a target peptide. This approach can be used to demonstrate the utility of the mIgE-specific peptide immunogen for the immunotherapy of allergic disease either by passive immunization with monoclonal antibodies, or by active immunization[6,7].

The feasibility of using such a peptide-KLH vaccine to provide immunotherapy to patients with IgE-mediated sensitivities has been reported by Stanworth et al. using a different ε-chain peptide[12,13]. A rabbit anti-peptide serum, selected from bleeds obtained from multiple immunizations which produced better-than-average anti-peptide tit r, reduced induced histamine release from rat peritoneal mast cells in a titer-dependent fashion. This

inhibitory activity was further confirmed by *in vivo* tests in a rat passive cutaneous anaphylaxis (PCA) model. The effect of this rabbit anti-peptide serum on anaphylaxis was assessed by measurement of the area of blueing and by an estimate of the color intensity when given to rats which had been previously sensitized by multiple allergen application prior to anaphylactic challenge with allergen. These results were preliminary indications for the feasibility of using peptide-based vaccines for the treatment of allergy. However, this peptide-conjugate strategy has met with considerable difficulties. For example, it has been found that the resultant clones and antisera raised by such conjugates contain more antibodies directed at the epitopes on the protein carrier, KLH, than to the target-peptide[14]. Other major deficiencies of protein carrier conjugate vaccines include: less-than-optimal immune stimulatory capability, manufacturing difficulties stemming from the poorly defined composition of the carrier protein, and the non-uniformity of the conjugation reaction.

Those skilled in the art usually use a conjugated protein carrier for synthetic peptide immunogens because small peptides are poor immunogens. In order to be rendered immunogenic, these peptides are usually conjugated to large carrier proteins chemically or by gene fusion. These processes, however, can produce unpredictable conformational changes in peptides. Moreover, the immune response is frequently misdirected to the immunodominant carrier. Consequently, the development of a potent vaccine that provides long-lasting protection from allergies awaits further immunogen design. Careful explorations into the design of "optimal immunogens," including full validation by the results of extensive experimental trials, still remain to be accomplished.

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OBJECTS OF THE INVENTION

It is an objective of the present invention to employ a group of membrane-bound IgE (mIgE) ε-chain peptide immunogens chemically synthesized linearly in tandem with T helper epitope peptides, which when introduced to mammals, including humans, can elicit high titer antibodies to exposed peptide sites of the membrane anchoring domain of human mIgE.

Another objective is to design optimal peptide immunogens, with specific amino acid sequences taken from the human mIgE heavy chain membrane anchoring domain (SEQ ID NOS:1,2) and attached to peptides containing promiscuous human helper T cell epitopes in specified orientations which, when introduced into mammals, including humans, will stimulate production of efficacious antibodies to the sites on human mIgE anchor domain. These antibodies may result in the reduction in IgE-producing B lymphocytes and thereby attenuate allergen-induced IgE production, constrain mast cell activation by IgE-allergen complexes, reduce the consequent release of chemical mediators such as histamines responsible for allergic symptoms and depress IgE-mediated passive cutaneous anaphylaxis (PCA). The end result is expected to be the reduction of allergic symptoms.

Another objective is to develop an effective mIgE ε-chain peptide-based vaccine, employing compositions containing such linear peptide immunogens, so as to provide immunotherapy for the treatment of allergic reactions.

SUMMARY OF THE INVENTION

According to the present invention, a series of linearly arranged synthetic peptides which contain either of two peptide sequences corresponding to sites on the exposed portion of the membrane anchoring domain of human mIgE (SEQ ID NOS:1,2) or their immunog nic analogs thereof

together with a portion of a helper T-cell epitope (Th epitope) are made by solid phase synthesis. Compositions of the invention are used to immunize healthy mammals, e.g. rats and humans for the production of high titer antisera that is specific for the mIgE anchor membrane sites (SEQ ID NOS:1,2) and free of irrelevant antibodies.

According to the present invention, vaccines containing the synthetic peptide compositions as the key immunogen may also be prepared with an immunologically effective amount of linear synthetic peptide in the presence of a proper adjuvant and/or delivery vehicle. It is expected that such vaccine compositions will elicit a more focused anti-IgE peptide response than those of the peptide-carrier protein conjugates currently used by Chang et al.[4,6-8], thus providing better immunotherapy for the treatment of allergy.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to the use of a novel group of peptide-based immunogens for the generation of high titer antibodies to mIgE anchor membrane sites (SEQ ID NOS:1,2) on human IgE ε heavy chain in healthy mammals, including humans, ultimately for the treatment of IgE-mediated allergic diseases.

Table I shows the arrangement and the amino acid sequence of the Membrane Anchor Domain of the ε heavy chain of human membrane-bound IgE (mIgE), as deduced from the nucleic acid sequence of the predominant species of mRNA that codes for membrane-bound ε chain[7,8]. The regions on the ε chain sequence of the peptides used as the target immunogens of the invention are underlined: a single underline for SEQ ID NO:1, and a double underline for SEQ ID NO:2.

TABLE I

CH4 Domain | Membrane Anchor Domain/extracellular segment O...
ValAsnPro | GlyLeuAlaGlyGlySerAlaGlnSerGlnArgAlaProAspArgVal

LeuCysHisSerGlyGlnGlnGlnGlyLeuProArgAlaAlaGlyGlySerValProHisPro

/extracellular
ArgCysHisCysGlyAlaGlyArgAlaAspTrpProGlyProProGluLeuAspValCysVal

segment 1 /transmembrane anchor segment
GluGluAlaGluGlyGluAlaProTrpThrTrpThrGlyLeuCysIlePheAlaAlaLeuPhe

/cytoplasmic anchor segment
LeuLeuSerValSerTyrSerAlaAlaLeuThrLeuLeuMetValGlnArgPheLeuSerAla

ThrArgGlnGlyArgProGlnThrSerLeuAspTyrThrAsnValLeuGlnProHisAla

It is generally accepted by people skilled in the art that allergic symptoms, the immediate result of IgE-dependent hypersensitivities, are caused by the chemical mediators released by mast cells and basophils. This release is triggered when secretory IgE ligated to receptors on a mast cell or basophil is bound by the allergen for which the receptor-bound IgE is specific. The triggering event is actuated after the allergen binds to the the Fab' portion of the surface-bound IgE in an antigen-antibody type interaction. The allergen/antibody binding crosslinks the bivalent surface-bound IgE and induces conformational changes in the distal Fc region of IgE, the region of IgE in direct contact with a high affinity Fc receptor on the mast/basophil cell surface as well as receptor site(s) on the cell surface. By a mechanism as yet not precisely known, the conformational changes activate the cell-IgE-allergen complex with the resultant release from the cell of chemical mediators, including histamine, inducing allergic symptoms and the further secretion of IgE. The secretory IgE which mediates the allergic reaction is produced by terminally differentiated B cells in response to allergen.

Besides secreting the circulatory IgE which becomes bound to mast cells and basophils, B cells committed to IgE synthesis also display membrane-bound IgE (mIgE) on their surface. The mIgE molecules are allergen receptors and are believed to play regulatory roles in the maturation of the B cells, and in activation of the B cells by allergen-specific T cells. The mIgE is distinguishable from the secreted IgE by a membrane-anchoring segment which extends from the C-terminus of the heavy chains which serves to attach the mIgE to the cell membrane. By determining the nucleotide sequences of pertinent segments in human genomic DNA and in mRNA from human mIgE-expressing B cells, amino acid sequences for two immunogenic sites on the extracellular portion of the

anchor domain were deduced (SEQ ID NOS:1,2). These sites are present on alternative forms of mIgE that result from different mRNA splicing events. Both sites are present on the predominant species of membrane-bound ε-chain, in the orientation shown in Table I. The presence and specificity of these sites as well as their accessibility to antibodies were confirmed with specific antibodies[4,8]. The presence of anti-IgE antibodies directed against such specific mIgE sites, through either active or passive immunization, can lead to the reduction in the numbers of IgE-producing B cells and a concomitant reduction in circulating IgE, possibly through the lytic removal of the IgE-expressing cells[10,11]. Moreover, it is desirable to target anti-IgE antibodies to the membrane-anchor domain because the domain is a surface marker specific to IgE-expressing cells. This target site is not available on secreted IgE. Thus, such anti-mIgE cannot bind and crosslink IgE bound to receptors on mast cells and basophils, and cannot by itself induce histamine release. The removal of IgE-expressing cells in hosts suffering from allergic reactions may result in the down-regulation of IgE production and have a therapeutic outcome.

Such interventions employed in the treatment of allergy through the use of specific anti-IgE antibodies, i.e. a kind of immunotherapy, can be achieved either passively, through prophylactic treatment with specific "site-directed" antibodies to IgE, or, more preferably, actively, by providing the host with a vaccine comprised of site-directed peptide immunogens, to elicit the production by the host of its own site-directed anti-IgE antibodies. It is believed that active immunization will provide a more effective and longer lasting form of protection.

The sites on the extracellular segment of the membrane anchor domain (SEQ ID NOS:1,2), arranged on membrane-bound IgE as shown in Table I, have been

confirmed as immunogenic sites that are acessible to antibodies through the cross-reactivities of the surface of IgE-secreting cells to antibodies that had been generated by immunizing animals with mIgE anchor membrane peptides coupled to a carrier protein, keyhole limpet hemocyanin (KLH)[4,5].

A major deficiency of these prototype "mIgE peptide" vaccines are their weak immunogenicities, an inherent problem associated with almost all self-antigens. In the present invention, specific examples are provided for the linkage of synthetic immunostimulatory elements to the mIgE peptides (SEQ ID NOS:1,2) in specified orientations such that potent antibodies directed to these sites on mIgE can be broadly generated in a genetically diverse host population. In turn, these antibodies may lead to reduction of IgE-expressing cells, reduced levels of circulating IgE, and diminished IgE-mediated responses, thus resulting in an effective treatment for the prevention of IgE-mediated allergic diseases.

The peptide immunogens of the current invention, while being substantially incapable of mediating non-cytolytic histamine release, are capable of eliciting antibodies with serological cross-reactivity with the target amino acid sequences of the extracellular region of the human mIgE membrane anchor domain (SEQ ID NOS:1,2).

The initial dose, e.g. 0.2-2.5 mg; preferably 1 mg, of immunogen is to be administered by injection, preferably intramuscularly, followed by repeat (booster) doses. Dosage will depend on the age, weight and general health of the patient as is well known in the vaccine and therapeutic arts.

While there is no particular limitation to species of mammals provided for the preparation of antibodies, it is generally preferred to use mice, rabbits, guinea pigs, pigs, goats, rats or sheep, etc. as the hosts.

For active immunization, the term "immunogen" referred to herein relates to a synthetic peptide which is capable of inducing antibodies against the mIgE membrane anchor domain (SEQ ID NOS:1,2), leading to suppression of IgE-mediated basophil and mast cell degranulation. The immunogen of the present invention includes linear synthetic peptides which contain promiscuous helper T cell epitopes (Th epitopes). The Th peptides are covalently attached to the mIgE membrane anchor domain peptide (SEQ ID NOS:1,2), with a spacer, so as to be adjacent to either the N- or C-terminus of the membrane anchor peptides, in order to evoke efficient antibody responses. The immunogen may also comprise an immune stimulatory amino acid sequence corresponding to a domain of an invasin protein from the bacteria *Yersinia* spp[15]. The invasin domain is attached through a spacer to a Th peptide.

The immunogen of the present invention minimizes the generation of irrelevant antibodies to elicit a more focused immune response to the "target sequences", the desired reactivity to mIgE membrane anchor sites (SEQ ID NOS:1,2), without producing undesirable side effects which may complicate the immunotherapy process for the treatment of allergy. However, when the desired target sequence is short, such as the 26 and 17 amino acid mIgE peptides (SEQ ID NOS:1,2) of the present invention, one faces other challenges because such short peptide antigens are usually T cell-dependent antigens, i.e. the presence of a T helper epitope is required to render such antigens immunogenic. Much effort had to be expended in the design of linear synthetic immunogens containing the short mIgE anchor membrane peptides (SEQ ID NOS:1,2) to provide for functional T-cell epitopes.

The peptides of this invention are represented by the formulas

$$(A)_n\text{-}(Th)_m\text{-}(B)_o\text{-}(\text{mIgE peptide})$$

wherein:

A is an amino acid, α-NH_2, a fatty acid or a derivative thereof, or an invasin domain;
B is an amino acid;
Th is a helper T cell epitope or an immune enhancing analog or segment thereof; mIgE peptide is:
Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu (SEQ ID NO:1);

or

Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID NO:2);
or an immunogenic analog thereof;
n is from 1 to about 10;
m is from 1 to about 4; and
o is from 0 to about 10.

The peptide immunogen of the present invention comprises from about 20 to about 100 amino acid residues, preferably from about 20 to about 50 amino acid residues and more preferably from about 20 to about 35 amino acid residues.

When A is an amino acid, it can be any non-naturally occurring or any naturally occurring amino acid. Non-naturally occurring amino acids include, but are not limited to, ß-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine, γ-amino butyric acid, homoserine, citrulline and the like. Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Moreover, when m is greater than one, and two or more of the A groups are amino acids, then each amino acid may be independently the same or different.

When A is a fatty acid, such as stearic acid, palmitic acid or a fatty acid derivative, such as tripalmitoyl cysteine (Pam_3Cys), it acts as an adjuvant by enhancing the immune stimulating properties of the Th epitope[16]. The fatty acid moiety or its derivative is preferably located at the amino terminus of the mIgE peptide. When A is a fatty acid or its derivative, the peptide immuogen comprises 2 or 3 additional A moieties which are amino acids. As used herein, the fatty acid is selected from the group with a hydrocarbon chain of 8 to 24 carbon atoms. The hydrocarbon chain can be saturated or unsaturated.

When A is an invasin domain, it is an immune stimulatory epitope from the invasin protein of a *Yersinia* species. This immune stimulatory property results from the capability of this invasin domain to interact with the ß1 integrin molecules present on T cells, particularly activated immune or memory T cells. The specific sequence for an invasin domain found to interact with the $\beta 1$ integrins has been decribed by Brett et al [15], incorporated herein by reference. In a preferred embodiment, the invasin domain (Inv) for linkage to a promiscuous Th epitope has the sequence:

Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-Thr-Tyr-Gln-Phe (SEQ ID NO:3)

or is an immune stimulatory analog thereof from the corresponding region in another *Yersinia* species invasin protein. Such analogs thus may contain substitutions, deletions or insertions of amino acid residues to accommodate strain to strain variation, provided that the analogs retain immune stimulatory properties.

In one embodiment, n is 1 and A is α-NH_2. In another embodiment, n is 4 and A is α-NH_2, an invasin domain (Inv), glycine and glycine, in that order.

B is a spacer and is an amino acid which can be naturally occurring or the non-naturally occurring amino

acids as described above. Each B is independently the same or different. In addition, the amino acids of B can form a flexible hinge, or spacer, to enhance the immune response to the Th epitope and mIgE peptides and their immunogenic analogs thereof. Examples of sequences encoding flexible hinges are found in the immunoglobulin heavy chain hinge region. Flexible hinge sequences are often proline rich. One particularly useful flexible hinge is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO:4), where Xaa is any amino acid, and preferably aspartic acid.

Immunogenicity can also be improved through the addition of spacer residues (e.g. Gly-Gly) between the promiscuous Th epitope and the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof. In addition to physically separating the Th epitope from the B cell epitope, i.e., the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunological analogs thereof, the spacer glycine residues can disrupt any artifactual secondary structures created by the joining of the Th epitope with the the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof and thereby eliminate interference between the T and/or B cell responses. The conformational separation between the helper cell and the antibody eliciting domains thus permits more efficient interactions between the presented immunogen and the appropriate Th and B cells.

Th is a sequence of amino acids (natural or non-natural amino acids) that comprises a Th epitope. A Th epitope can consist of a continuous or discontinuous epitope. Hence not every amino acid of Th is necessarily part of the epitope. Accordingly, Th epitopes, including analogs and segments of Th epitopes, are capable of enhancing or stimulating an immune response to the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunological analogs thereof. Th epitopes that are

immunodominant and promiscuous are highly and broadly reactive in animal and human populations with widely divergent MHC types[17-19]. The Th domain of the subject peptides has from about 10 to about 50 amino acids and preferably from about 10 to about 30 amino acids. When multiple Th epitopes are present (i.e. m ≥ 2), then each Th epitope is independently the same or different.

Th epitope analogs include substitutions, additions, deletions and insertions of from one to about 10 amino acid residues in the Th epitope. Th segments are contiguous portions of a Th epitope that are sufficient to enhance or stimulate an immune response to the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunological analogs thereof.

Th epitopes of the present invention include hepatitis B surface and core antigen helper T cell epitopes (HB_sTh and HB_cTh), pertussis toxin helper T cell epitopes (PT Th), tetanus toxin helper T cell epitopes (TT Th), measles virus F protein helper T cell epitopes (MV_F Th), *Chlamydia trachomatis* major outer membrane protein helper T cell epitopes (CT Th), diphtheria toxin helper T cell epitopes (DT Th), *Plasmodium falciparum* circumsporozoite helper T cell epitopes (PF Th), *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes (SM Th), *Escherichia coli* TraT helper T cell epitopes (TraT Th) and immune-enhancing analogs and segments of any of these Th epitopes. Examples of Th epitope sequences are provided below as Table II:

TABLE II

HB_1 Th: Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp (SEQ ID NO:5)

PT_1 Th: Lys-Lys-Leu-Arg-Arg-Leu-Leu-Tyr-Met-Ile-Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu-Gln-Tyr-Tyr-Asp-Tyr (SEQ ID NO:6)

TT_1 Th: Lys-Lys-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu (SEQ ID NO:7)

TT_2 Th: Lys-Lys-Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu (SEQ ID NO:8)

PT_{1A} Th: Tyr-Met-Ser-Gly-Leu-Ala-Val-Arg-Val-His-Val-Ser-Lys-Glu-Glu (SEQ ID NO:9)

TT_3 Th: Tyr-Asp-Pro-Asn-Tyr-Leu-Arg-Thr-Asp-Ser-Asp-Lys-Asp-Arg-Phe-Leu-Gln-Thr-Met-Val-Lys-Leu-Phe-Asn-Arg-Ile-Lys (SEQ ID NO:10)

PT_2 Th: Gly-Ala-Tyr-Ala-Arg-Cys-Pro-Asn-Gly-Thr-Arg-Ala-Leu-Thr-Val-Ala-Glu-Leu-Arg-Gly-Asn-Ala-Glu-Leu (SEQ ID NO:11)

MV_{F1} Th: Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID NO:12)

HB_c Th: Val-Ser-Phe-Gly-Val-Trp-Ile-Arg-Thr-Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu (SEQ ID NO:13)

MV_{F2} Th: Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-Thr-His-Val-Asp-Thr-Glu-Ser-Tyr (SEQ ID NO:14)

TT_4 Th: Trp-Val-Arg-Asp-Ile-Ile-Asp-Asp-Phe-Thr-Asn-Glu-Ser-Ser-Gln-Lys-Thr (SEQ ID NO:15)

TT_5 Th: Asp-Val-Ser-Thr-Ile-Val-Pro-Tyr-Ile-Gly-Pro-Ala-Leu-Asn-Ile-Val (SEQ ID NO:16)

CT Th: Ala-Leu-Asn-Ile-Trp-Asp-Arg-Phe-Asp-Val-Phe-Cys-Thr-Leu-Gly-Ala-Thr-Thr-Gly-Tyr-Leu-Lys-Gly-Asn-Ser (SEQ ID NO:17)

DT_1 Th: Asp-Ser-Glu-Thr-Ala-Asp-Asn-Leu-Glu-Lys-Thr-Val-Ala-Ala-Leu-Ser-Ile-Leu-Pro-Gly-Ile-Gly-Cys (SEQ ID NO:18)

DT_2 Th: Glu-Glu-Ile-Val-Ala-Gln-Ser-Ile-Ala-Leu-Ser-Ser-Leu-Met-Val-Ala-Gln-Ala-Ile-Pro-Leu-Val-Gly-Glu-Leu-Val-Asp-Ile-Gly-Phe-Ala-Ala-Thr-Asn-Phe-Val-Glu-Ser-Cys (SEQ ID NO:19)

PF Th: Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser (SEQ ID NO:20)

SM Th: Lys-Trp-Phe-Lys-Thr-Asn-Ala-Pro-Asn-Gly-Val-Asp-Glu-Lys-Ile-Arg-Ile (SEQ ID NO:21)

$TraT_1$ Th: Gly-Leu-Gln-Gly-Lys-His-Ala-Asp-Ala-Val-Lys-Ala-Lys-Gly (SEQ ID NO:22)

$TraT_2$ Th: Gly-Leu-Ala-Ala-Gly-Leu-Val-Gly-Met-Ala-Ala-Asp-Ala-Met-Val-Glu-Asp-Val-Asn (SEQ ID NO:23)

$TraT_3$ Th: Ser-Thr-Glu-Thr-Gly-Asn-Gln-His-His-Tyr-Gln-Thr-Arg-Val-Val-Ser-Asn-Ala-Asn-Lys (SEQ ID NO:24)

The linear synthetic peptides of this invention, as described by the formulas $(A)_n$-$(Th)_m$-$(B)_o$-(mIgE peptide) or (mIgE peptide)-$(B)_o$-$(Th)_m$-$(A)_n$, have the Th epitope covalently attached through spacer B to the N terminus of either of the the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof. In a preferred embodiment the Th epitope is HB_s Th, PT_1 Th, PT_2 Th, TT_1 Th, TT_3 Th, or MV_{F1} Th.

The sequence of the mIgE membrane anchor peptide comprises:

Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-
Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-
Gly-Gln-Gln-Gln-Gly-Leu (SEQ ID NO:1);

or

Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-
Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID NO:2).

Immunogenic peptide analogs of the mIgE anchor peptides (SEQ ID NOS:1,2) according to the invention, may further comprise substitutions, additions, deletions, or insertions of from one to about four amino acid residues provided that the peptide analogs are capable of eliciting immune responses crossreactive with the mIgE membrane anchor peptides (SEQ ID NOS:1,2). The substitutions, additions, and insertions can be accomplished with natural or non-natural amino acids as defined herein.

Accordingly, preferred peptide immunogens of this invention are the linear synthetic peptides containing the mIgE membrane anchor peptides (SEQ ID NOS:1,2) or immunological analogs thereof and Th. The more preferred peptide immunogens are those linear constructs containing the mIgE membrane anchor peptides (SEQ ID NOS:1,2) or immunogenic analogs thereof; a spacer (e.g Gly-Gly); a Th epitope selected from the group consisting of HB_s Th, PT_1 Th, PT_2 Th, TT_1 Th, TT_3 Th, and MV_{F1} Th (SEQ ID NOS:5,6,11,7,10,12, respectively); and, optionally, an Inv domain (SEQ ID NO:3) or analog thereof.

The peptide immunogens of this invention can be made by chemical synthesis methods which are well known to the ordinarily skilled artisan. See, for example, Grant, ed. Synthetic Peptides[20]. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α-NH_2 protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431.

When A is a fatty acid, it can be easily added to the N-terminal amino group of the synthesized peptide by the well known dicyclohexyl-carbodiimide coupling method.

Pam_3Cys, lipoamino acid S-[2,3-Bis(palmitoyloxy)-(2R)-propyl-N-palmitoyl-(R)-cysteine may also be synthesized by chemical methods. Pam_3Cys can be coupled to, for example, the N terminus of the mIgE peptide by solid-phase synthesis using Pam_3Cys-OH in the final coupling step to link the lipoamino acid to a resin-bound mIgE peptide chain. To improve the solubility of the final coupled lipopeptide product, the solid-phase peptide can be elongated with additional serine and lysine residues at the N-terminus.

After complete assembly of the desired immunogen, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the functional groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well known to one of ordinary skill in the art.

Other chemical means to generate the linear peptide constructs of the invention containing mIgE and Th sites include the ligation of the haloacetylated and the cysteinylated peptide through the formation of a

"thioether" linkage. For example, a cysteine can be added to the C terminus of a Th-containing peptideand the thiol group of cysteine may be used to form a covalent bond to an electrophilic group such as an N^{α} chloroacetyl-modified or a maleimide-derivatized α- or ϵ-NH_2 group of a lysine residue attached to the N-terminus of an mIgE membrane anchor peptide (SEQ ID NOS:1 or 2) or immunogenic analogs thereof. In this manner, a construct with Th-(mIgE peptide) or its reverse, (mIgE peptide)-Th, may be obtained.

The subject immunogen may also be polymerized. Polymerization can be accomplished for example by reaction between glutaraldehyde and the -NH_2 groups of the lysine residues using routine methodology. By another method, the linear "A-Th-spacer-(mIgE peptide)" or "(mIgE peptide)-spacer-$(Th)_m$-$(A)_n$" immunogen can be polymerized or co-polymerized by utilization of an additional cysteine added to the N-terminus of the linear "A-Th-spacer-(mIgE peptide) or "(mIgE peptide)-spacer-$(Th)_m$-$(A)_n$" immunogen. The thiol group of the N-terminal cysteine can be used for the formation of a "thioether" bond with haloacetyl-modified amino acid or a maleimide-derivatized α- or ϵ-NH_2 group of a lysine residue that is attached to the N-terminus of a branched poly-lysyl core molecule (e.g., K_2K, K_4K_2K or $K_8K_4K_2K$).

Alternatively, the longer linear peptide immunogens can be synthesized by well known recombinant DNA techniques. Any standard manual on DNA technology provides detailed protocols to produce the peptides of the invention. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated into a nucleic acid sequence, and preferably using optimized codon usage for the organism in which the gene will be expressed. Next, a synthetic gene is made, typically by synthesizing overlapping oligonucleotides which ncode the peptide and any regulatory el m nts, if

necessary. The synthetic gene is inserted in a suitable cloning vector and recombinants are obtained and characterized. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The efficacy of the immunogen of the present invention can be established by injecting an animal, for example, rats, followed by monitoring the humoral immune response to the mIgE membrane anchor peptides (SEQ ID NOS:1,2) and immunogenic analogs thereof, as detailed in the Examples.

Another aspect of this invention provides a vaccine composition comprising an immunologically effective amount of one or more of the immunogens of this invention in a pharmaceutically acceptable delivery system. Such vaccine compositions are used for prevention of atopic allergic reactions including allergic rhinitis, those of food allergies, asthma, anaphylaxis, and other IgE-mediated hypersensitive reactions such as virally-induced asthma.

Accordingly, the subject peptides can be formulated as a vaccine composition using adjuvants, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions. Such formulations are readily determined by one of ordinary skill in the art and include formulations for immediate release and/or for sustained release, and for induction of systemic immunity and/or induction of localized mucosal immunity, which may be accomplished by, for example, immunogen entrapment by microparticles. The present vaccines can be administered by any convenient route including subcutaneous, oral, intramuscular, or other parenteral or enteral route. Similarly the vaccines can be administered as a single dose or multiple doses. Immunization sch dules are readily determined by the

ordinarily skilled artisan. Adjuvants or emulsifiers that can be used in this invention include alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, L121, emulsigen and ISA 720 as well as the other efficacious adjuvants and emulsifiers.

The vaccine composition of the instant invention contain an effective amount of one or more of the immunogens of the present invention and a pharmaceutically acceptable carrier. Such a composition in a suitable dosage unit form generally contains about 0.5 μg to about 1 mg of the immunogen per kg body weight. When delivered in multiple doses, it may be conveniently divided into an appropriate amount per dosage unit form.

Vaccines which contain cocktails of the subject immunogens with two or more of the Th epitopes may enhance immunoefficacy in a broader population and thus provide an improved immune response to the mIgE membrane anchor peptide (SEQ ID NOS:1,2). For example, a cocktail of Peptide Nos. 1-4 of Example 1 and 7-10 of Example 4 is useful. Other immune stimulatory synthetic peptide-based mIgE anchor peptide immunogens are arrived at through modification into lipopeptides, such as Pam_3Cys, so as to provide a built-in adjuvant for a potent vaccine. The immune response to synthetic mIgE anchor peptide-containing immunogens can be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan *et al*[21]. The immunogens can be encapsulated with or without an adjuvant, including covalently attached Pam_3Cys, and such microparticles can carry an immune stimulatory adjuvant such as Freund's Incomplete Adjuvant or alum. The microparticles function to potentiate immune responses to an immunogen, including localized mucosal immunity which may be especially applicable to mucosally localized allergic reactions, and to provide time-controlled release for sustained or

periodic responses, for oral administration, and for topical administration[21,22].

Specific peptide immunogens and compositions are provided in the following examples to illustrate the invention. The peptide immunogen of the invention can be useful for the amelioration of IgE-mediated allergic disorders.

EXAMPLE 1

SYNTHESIS OF PEPTIDE IMMUNOGENS NOS 1-4 "Th-SPACER-(mIgE PEPTIDE)" AND "(mIgE PEPTIDE)-SPACER-Th"

Immunogens 1-4 (Table III) were synthesized by solid phase synthesis using F-moc chemistry on an Applied Biosystems Peptide Synthesizer Model 430A or 431 according to manufacturer's instructions. After complete assembly of the peptide, the resin was treated according to standard procedures to cleave the peptide from the resin and deprotect the functional groups on amino acid side chains. The free peptide was then purified by HPLC and characterized biochemically for amino acid content and sequence.

The structures of Peptide immunogens Nos. 1-4, from the amino terminus to the carboxyl terminus, are symbolized as A-Th-B-(mIgE peptide) or (mIgE peptide)-B-Th-A, where "A" is NH_2-, "B" is a Gly-Gly spacer, "Th" is the measles virus F1 helper T cell epitope MV_{F1} Th (SEQ ID NO:12), and "(mIgE peptide)" is either mIgE peptide of SEQ ID NO:1 or mIgE peptide of SEQ ID NO:2. Thus, Peptide No. 1 may be more explicitly represented as "MV_{F1} Th-GG-(mIgE 1)" and Peptide No. 2 as "(mIgE 2)-GG-MV_{F1} Th", for examples. The actual sequences for Peptides 1-4 (SEQ ID NOS:25-28) are shown in Table 2.

TABLE 2

Amino Acid Sequences of Peptide Nos. 1-4

Peptide	Sequence
1. MV_{F1}Th-GG-mIgE1	Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Gly-Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu (SEQ ID No:25)
2. mIgE1-GG-MV_{F1}Th	Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu-Gly-Gly-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID No:26)
3. MV_{F1}Th-GG-mIgE2S	Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-Gly-Gly-Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID No:27)
4. mIgE2-GG-MV_{F1}Th	Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr-Gly-Gly-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val (SEQ ID No:28)

EXAMPLE 2

IMMUNIZATION OF RATS WITH "Th-SPACER-(mIgE PEPTIDE)" AND "(mIgE PEPTIDE)-SPACER-Th" LINEAR CONSTRUCTS, PEPTIDE NOS. 1-4, AND EVALUATION OF IMMUNOGENICITY BY ELISA

Efficacy of Peptide immunogens Nos. 1-4 is evaluated on groups of five rats by the experimental immunization protocol outlined below and by serological assay for determination of immunogenicity.

Experimental Design:

Immunogen: Peptide Nos. 1-4 (1 per group)
Dose: 100 μg per immunization
Route: intramuscular
Adjuvant: Freund's Complete/Incomplete
Dose Schedule: week 0 (FCA), 3 and 6 weeks (IFA)
Bleed Schedule: weeks 0, 3, 6, 8, 10
Species: Sprague-Dawley rats
Group size: 5
Assay: ELISAs for anti-peptide activity.

Blood is collected and processed into serum, and stored prior to titering by ELISA with the target peptides (SEQ ID NOS:1,2).

Anti-peptide antibody activities are determined by ELISA (enzyme-linked immunosorbent assay) using 96-well flat bottom microtiter plates which are coated with the corresponding target mIgE peptide epitope as the immunosorbent, either Peptide 5 for mIgE anchor peptide site described by SEQ ID NO:1 or Peptide No. 6, described by SEQ ID NO:2. Aliquots (100 μL) of a peptide immunogen solution at a concentration of 5 μg/mL are incubated for 1 hour at 37°C. The plates are blocked by another incubation at 37°C for 1 hour with a 3% gelatin/PBS solution. The blocked plates are then dried and used for the assay. Aliquots (100 μL) of the test rat sera, starting with a 1:10 dilution in a sample dilution buffer and ten-fold serial dilutions thereafter, are added to the peptide coated plates. The plates are incubated for 1 hour at 37°C.

The plates are washed six times with 0.05% PBS/Tween® buffer. 100 μL of horseradish peroxidase labelled goat-anti-rat antibody is added at a dilution of 1:1,000 in conjugate dilution buffer (Phosphate buffer containing 0.5M NaCl, and normal goat serum). The plates are incubated for 1 hour at 37°C before being washed as

above. Aliquots (100 μL) of o-phenylenediamine substrate solution are then added. The color is allowed to develop for 5-15 minutes before the enzymatic color reaction is stopped by the addition of 50 μL 2N H_2SO_4. The A_{492nm} of the contents of each well is read in a plate reader.

All sera are assayed by anti-peptide ELISA and those samples which give A_{492nm} values of ≥ 0.2 at a 1:100 dilution are recorded as seropositive. Normal rat serum is used as a negative control. Results are also compared to positive control serum from rats immunized with KLH conjugates of either Peptide No. 5 or 6 described above, to demonstrate improved immunogenicity for peptide of the invention.

EXAMPLE 3

EVALUATION OF RAT ANTI-SERA TO PEPTIDE NOS. 1-4 FOR FUNCTIONAL EFFICACY ON HUMAN IgE-SECRETING CELL LINES

The peptide immunogen compositions of this invention evoke antibodies that target IgE-secreting human B cells and inhibit IgE production. Unlike most antibodies to IgE, the mIgE peptide-elicited antibodies do not bind to cells bearing only the secretory form of IgE bound to receptors and are therefore incapable, by themselves, of triggering the release of the chemical mediators of allergic response from IgE-sensitized mast cells and basophils. These biological activities are of relevance to immunotherapy for allergy and can be observed in the rat antisera to Peptide immunogens Nos. 1-4 by assaying for the following functions:

1. Antibody binding to IgE-secreting B cells and not binding to IgE-sensitized basophils. A human IgE-producing B cell line, for example the myeloma cell line SKO-007 (ATCC, Rockville MD) or an EBV-transformed B cell line 8866, is incubated with serial dilutions of the rat anti-mIgE and antibody binding is detected using FITC-

labeled ant-rat IgG and quantitated by fluorescence flow cytometry[4]. The extent of binding by the rat anti-mIgE antibodies to normal human basophils, prepared from peripheral blood and loaded or sensitized with secreted IgE, is evaluated in a similar fashion[5].

2. Reduction in the IgE accumulation of IgE-secreting cells. IgE accumulates in the culture medium of myeloma cell line SKO-007 and in like IgE-secreting cell lines. To determine whether treatment of IgE-secreting cells with the rat anti-mIgE sera elicited by Peptide immunogens Nos. 1-4 results in a decrease of IgE secreted into the medium, the cells are treated with a range of antibody concentrations, and IgE levels in the medium are monitored over time by IgE-specific ELISA[11]. Efficacious antibodies result in a dose-related reduction in accumulated IgE.

3. Lysis of IgE-secreting cells by antibody dependent cell-mediated cytotoxicity (ADCC). The capability of a range of concentrations of the rat anti-mIgE sera to induce cytolysis of SKO-007 cells, or the like, by ADCC activity is evaluated for percent lysis by an assay employing effector cells from several donors[11].

4. Inability to induce histamine release from basophils. Rat anti-mIgE sera is used at several concentrations to show a dose-related ability to induce histamine release from IgE-sensitized isolated peripheral blood basophils. Histamine-release is quantitated by fluorometric assay[5]. An inability to induce histamine release is evidence for the efficacy and safety of the peptide immunogen of the present invention.

EXAMPLE 4

COCKTAIL OF PEPTIDE IMMUNOGENS TO FURTHER BROADEN THE RESPONSIVE POPULATION

A cocktail of immunogenic Th-spacer-(mIgE peptide) and (mIgE peptide)-spacer-Th peptides wherein there are more than one Th epitope for recognition by diverse MHC types can serve to broaden immune responsiveness in a genetically diverse human population. Promiscuous Th epitopes useful for this purpose are selected from Table II. The Th epitopes of Table II that are useful for such peptide cocktails include but are not limited to the MV_{F1} Th peptide (SEQ ID NO:12) used in Peptide Nos. 1-4 (SEQ ID NOS:25-28) and the HB_s Th peptide (SEQ ID NO:5). Peptides containing either of the two anchor membrane mIgE peptide sequences (SEQ ID NOS:1 or 2) and the HB_s Th peptide are described in Table IV as Peptide immunogens Nos. 7-10 (SEQ ID NOS: 29-32).

Peptide immunogens Nos. 7-10 are synthesized as described in Example 1 and combined with each other and with Peptide immunogens Nos. 1-4, in equal molar ratios, to formulate into a peptide cocktail. A composition of the invention formulated as a cocktail is evaluated for immunopotency in rats by the protocol described below.

Experimental Design:

Immunogens: (1) Cocktail : Peptide Nos. 1-4 and 7-10

(2) Positive Control: Individual KLH conjugates of Peptide Nos. 5 and 6 combined 1:1 (One immunogen per group of rats)

Dose: Molar equivalents of each synthetic peptide immunogen or mIgE peptide equivalent, to equal 100 μg total or 12.5 μg of ach peptide per immunization

Route: intramuscular
Adjuvants: (1) Freund's Complete/Incomplete
(2) 0.4% Alum (Aluminum hydroxide)
(One of either adjuvant per immunogen per group).
Dose Schedule: week 0, 2 and 4 weeks
CFA/IFA groups receive CFA week 0, IFA weeks 2 and 4. Alum groups receive Alum formulations for all 3 doses)
Bleed Schedule: weeks 0, 3, 6 and 8
Species: Sprague-Dawley rats
Group size: 5, 4 groups
Assay: 2 ELISAs for anti-peptide activity, solid-phase substrates are Peptide Nos. 5 and 6 (SEQ ID NOS:1,2)

Blood is collected, processed into serum and stored prior for determination of seroconversion by the two anti-peptide ELISAs as described in Example 2.

This study is designed to demonstrate improved immunogenicity for this embodiment of the peptide invention, and to demonstrate efficacy for a composition of the invention formulated with a pharmaceutically acceptable adjuvant, Alum.

TABLE IV

Amino Acid Sequences of Peptide Nos. 7-10

Peptide	Sequence
7. HB_1Th-GG-mIgE1	Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp-Gly-Gly-Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu (SEQ ID No:29)
8. mIgE1-GG-HB_1Th	Leu-Ala-Gly-Gly-Ser-Ala-Gln-Ser-Gln-Arg-Ala-Pro-Asp-Arg-Val-Leu-Cys-His-Ser-Gly-Gln-Gln-Gln-Gly-Leu-Gly-Gly-Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp (SEQ ID No:30)
9. HB_1Th-GG-mIgE2	Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp-Gly-Gly-Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr (SEQ ID No:31)
10. mIgE2-GG-HB_1Th	Pro-Glu-Leu-Asp-Val-Cys-Val-Glu-Glu-Ala-Glu-Gly-Glu-Ala-Pro-Trp-Thr-Gly-Gly-Phe-Phe-Leu-Leu-Thr-Arg-Ile-Leu-Thr-Ile-Pro-Gln-Ser-Leu-Asp (SEQ ID No:32)

EXAMPLE 5

CLINICAL TRIAL TO DEMONSTRATE THERAPEUTIC EFFICACY OF COCKTAIL

Individual constructs carrying Th peptide sequences from measles virus F and hepatitis B surface antigen are promiscuous for multiple human HLA DR antigens, so as to provide maximum immunogenicity in a genetically diverse human population. Moreover, because these Th peptides are derived from children's vaccines, childhood vaccinations are a potential source of Th memory in an immunized human population. Thus, children's vaccines have the potential to afford enhanced immunopotency to anti-allergy vaccines comprised of mixtures of such Th peptides. The clinical protocol below is designed to demonstrate efficacy for compositions of the invention formulated as a mixture of such linear "Th-Spacer-(mIgE Peptide)" and "(mIgE Peptide)-spacer-Th" peptide constructs, in a widely acceptable adjuvant, Alum.

Experimental Design:

Subjects:	Hay fever patients
Season & Duration:	Hay fever seasons, 8 weeks
Groups:	4 groups, 1 group/immunogen/dose N=15 per group, 12 receive immunogen, 3 receive placebo
Immunogen:	Cocktail 1; Peptide Nos. 1-4, 7-10
Adjuvant:	0.2% Alum
Dose:	Molar equivalents of each synthetic peptide to equal 500 μg total or 62.5 μg of each peptide per dose
Route:	intramuscular
Dose Schedule:	week 0, and 4 weeks
Evaluation schedule:	weeks 0, 4, and 6

Blood is collected, processed into serum, and stored prior to titering by ELISA as described in Example 2.

Efficacy and safety of the clinical composition are evaluated by comparisons of serological tests, skin test reaction, by recording patient usage of hay fever medication, by physical examination for allergic symptoms and adverse reactions, and by interviews to obtain subjective patient assessments of the product. Serological evaluations include the aforementioned ELISAs for antipeptide titers, and a standard automated spectrofluorimetric assay to determine reduction in histamine levels[13] as well as to ascertain that the products do not trigger histamine release. The skin test is an intradermal test in which a standardized solution of allergens is injected into the upper layers of the skin. Reactions to the allergens are quantitated in the skin test by determining the area of the typical "wheal and flare" produced in response to the allergens. The expected results include significant improvement in allergic symptoms at the endpoint of the study, and no evidence of histamine release.

This experiment demonstrates the clinical efficacy and safety of a pharmaceutically acceptable composition of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Walfield, Alan M.; Wang, Chang Yi

(ii) TITLE OF INVENTION: Synthetic IgE Membrane Anchor Peptide Immunogens for the Treatment of Allergy

(iii) NUMBER OF SEQUENCES: 32

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
1 5 10

Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
15 20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Pro Glu Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
1 5 10

Ala Pro Trp Thr
15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala Thr
1 5 10

Tyr Gln Phe
15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Pro Xaa Pro Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln
1 5 10

Ser Leu Asp
15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met Ser
1 5 10

Gly Leu Ala Val Arg Val His Val Ser Lys Glu Glu Gln
15 20 25

Tyr Tyr Asp Tyr
30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
1 5 10

Ile Thr Glu Leu
15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
1 5 10

Val Pro Lys Val Ser Ala Ser His Leu
15 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Met Ser Gly Leu Ala Val Arg Val His Val Ser Lys
1 5 10

Glu Glu
15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys Asp
1 5 10

Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn Arg Ile
15 20 25

Lys

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala Leu
1 5 10

Thr Val Ala Glu Leu Arg Gly Asn Ala Glu Leu
15 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
1 5 10

Gly Val
15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr
1 5 10

Arg Pro Pro Asn Ala Pro Ile Leu
15 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr
1 5 10

His Val Asp Thr Glu Ser Tyr
15 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu Ser
1 5 10

Ser Gln Lys Thr
15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Val Ser Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu
1 5 10

Asn Ile Val
15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr
1 5 10

Leu Gly Ala Thr Thr Gly Tyr Leu Lys Gly Asn Ser
15 20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val Ala
1 5 10

Ala Leu Ser Ile Leu Pro Gly Ile Gly Cys
15 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser Ser Leu
1 5 10

Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
15 20 25

Asp Ile Gly Phe Ala Ala Thr Asn Phe Val Glu Ser Cys
30 35

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser
1 5 10

Ser Val Phe Asn Val Val Asn Ser
15 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp Glu
1 5 10

Lys Ile Arg Ile
15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Leu Gln Gly Lys His Ala Asp Ala Val Lys Ala Lys
1 5 10

Gly

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp Ala
1 5 10

Met Val Glu Asp Val Asn
12

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr Arg
1 5 10

Val Val Ser Asn Ala Asn Lys
15 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
1 5 10

Gly Val Gly Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln
15 20 25

Arg Ala Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln
30 35

Gln Gly Leu
40

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
1 5 10

Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
15 20 25

Gly Gly Leu Ser Glu Ile Lys Gly Val Ile Val His Arg
30 35

Leu Glu Gly Val
40

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
1 5 10

Gly Val Gly Gly Pro Glu Leu Asp Val Cys Val Glu Glu
15 20 25

Ala Glu Gly Glu Ala Pro Trp Thr
30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Pro Gly Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
1 5 10

Ala Pro Trp Thr Gly Gly Leu Ser Glu Ile Lys Gly Val
15 20 25

Ile Val His Arg Leu Glu Gly Val
30

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
1 5 10

Leu Asp Gly Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln
15 20 25

Arg Ala Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln
30 35

Gln Gly Leu
40

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Leu Ala Gly Gly Ser Ala Gln Ser Gln Arg Ala Pro
1 5 10

Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu
15 20 25

Gly Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
30 35

Gln Ser Leu Asp
40

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
1 5 10

Leu Asp Gly Gly Pro Glu Leu Asp Val Cys Val Glu Glu
15 20 25

Ala Glu Gly Glu Ala Pro Trp Thr
30

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Pro Glu Leu Asp Val Cys Val Glu Glu Ala Glu Gly Glu
1 5 10

Ala Pro Trp Thr Gly Gly Phe Phe Leu Leu Thr Arg Ile
15 20 25

Leu Thr Ile Pro Gln Ser Leu Asp
30

We claim:

1. A peptide immunogen selected from the group consisting of:

$$(A)_n\text{-}(Th)_m\text{-}(B)_o\text{-}(\text{mIgE peptide})$$

or

$$(\text{mIgE peptide})\text{-}(B)_o\text{-}(Th)_m\text{-}(A)_n$$

wherein: A is an amino acid, α-NH_2, a fatty acid or a derivative thereof, or an invasin domain having immmune stimulatory property;

B is an amino acid;

Th is a helper T cell epitope, or an immune enhancing analog or segment thereof;

mIgE peptide is SEQ ID NO:1, SEQ ID NO:2, or an immunogenic analog thereof;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

2. The peptide immunogen of Claim 1 wherein said Th has an amino acid sequence selected from the group of sequences selected from SEQ ID NOS:5-24 or an immunogenic analog or segment thereof.

3. The peptide immunogen of Claim 1 having an amino acid sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32.

4. The peptide immunogen of Claim 2 further comprising a fatty acid.

5. The peptide immunogen of Claim 2 further comprising a fatty acid derivative.

6. The peptide immunogen of Claim 5 wherein the fatty acid derivative is Pam_3Cys.

7. The peptide immunogen of Claim 3 further comprising a fatty acid.

8. The peptide immunogen of Claim 3 further comprising a fatty acid derivative.

9. The peptide immunogen of Claim 8 wherein the fatty acid derivative is Pam_3Cys.

10. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 1 and a pharmaceutically acceptable delivery system.

11. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 2 and a pharmaceutically acceptable delivery system.

12. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 3 and a pharmaceutically acceptable delivery system.

13. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 4 and a pharmaceutically acceptable delivery system.

14. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 5 and a pharmaceutically acceptable delivery system.

15. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 6 and a pharmac utically acceptable delivery system.

16. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 7 and a pharmaceutically acceptable delivery system.

17. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 8 and a pharmaceutically acceptable delivery system.

18. A vaccine composition comprising an effective amount of a peptide immunogen of Claim 9 and a pharmaceutically acceptable delivery system.

19. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 1.

20. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 2.

21. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 3.

22. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 4.

23. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 5.

24. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 6.

25. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 7.

26. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 8.

27. A method of treating allergic reactions by administering to a patient an effective amount of a composition according to Claim 9.

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US95/13841

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 16/100, 16/46; A61K 39/00, 39/35
US CL : 530/324, 387.1, 403; 424/184.1, 185.1; 514/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 387.1, 403; 424/184.1, 185.1; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS & CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,260,416 (CHANG) 09 NOVEMBER 1993, see entire document.	1-27
Y	US, A, 5,231,026 (CHANG) 27 JULY 1993, see entire document.	1-27
Y	US, A, 5,281,699 (CHANG) 25 JANUARY 1994, see entire document.	1-27
Y	US, A, 5,274,075 (CHANG) 28 DECEMBER 1993, see entire document.	1, 9, 19
Y	US, A, 5,342,924 (CHANG) 30 AUGUST 1994, see entire document.	1, 9, 19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 FEBRUARY 1996	Date of mailing of the international search report 05 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T. Wessendorf Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

Inte...tional application No.
PCT/US95/13841

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE LANCET, Volume 336, issued 1990, Stanworth et al, "Allergy Treatment with a peptide vaccine", pages 1279-1281, see entire document.	1-27
A	The Journal of Immunology, volume 148, number 1, issued 01 January 1992, Peng et al, "A new isoform of human membrane bound IgE," pages 129-136, see entire document.	1-27
Y	Molecular Immunology volume 23, number 1, issued 1986, Stanworth et al, "Anti - e - chain antibodies as probes in the study of mast-cell triggering", pages 1231-1235, see entire document.	1-27
Y	European J. Immunology, volume 17, issued 1987, Leclerc et al, "A synthetic vaccine constructed by copolymerization of Band T cell determinants", pages 269-273, see entire document.	1-27
Y	US, A, 4,722,840 (VALENZUELA ET AL) 02 February 1988, see entire document.	1-27